linearality and low resolution of long DNA substrates in conventional TPM experiments. The FTPM method offers the best resolution (56 bp at 433 bp long DNA) in the presence of only a small stretching force (0.20 pN). We have used the FTPM method to investigate the RecBCD helicase motion along 4.1 kb long chi-containing duplex DNA molecules, and observed that translocation rate of RecBCD changes after chi sequence under limited ATP concentrations. This suggests that chi recognition by RecBCD does not require saturating ATP conditions, contrary to what have been previously reported.

2136-Pos Board B106
Kinetic Mechanism for Single stranded DNA binding and Translocation by *S. cerevisiae* Isw2
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The chromatin remodeling complex Isw2 from *S. cerevisiae* (yIsw2) mobilizes nucleosomes through an ATP-dependent reaction that is coupled to the translocation of the enzyme along intranucleosomal DNA. In this study we demonstrate that yIsw2 is capable of translocating along single-stranded DNA in a reaction that is coupled to ATP hydrolysis. We find that single-stranded DNA translocation by yIsw2 occurs through a series of repeating uniform steps with an overall macroscopic processivity of $P = (0.92 \pm 0.01)$; this processivity corresponds to an average translocation distance of (24 ± 4) nucleotides before dissociation. This processivity corresponds well to the processivity of nucleosome sliding by yIsw2 thus arguing that single-stranded DNA translocation may be fundamental to the double-stranded DNA translocation required for effective nucleosome mobilization by the enzyme. Furthermore, we find that a slow initiation process, following DNA binding, is required to make yIsw2 competent for DNA translocation. We also provide both evidence that this slow initiation process likely corresponds to the second step of a two-step DNA binding mechanism by yIsw2 and a quantitative description of the kinetics of this DNA binding mechanism.

2137-Pos Board B107
Single-molecule Measurements Of DnaB
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The replicative helicase of E. coli, DnaB, is a ring-shaped hexameric motor protein capable of unwinding double-stranded DNA (dsDNA) at a fork. It is thought to do this through ''steric occlusion,'' in which DnaB encircles and translocates along one single strand of DNA (ssDNA), forcing the other single strand (the occluded strand) to pass outside the ring. Using magnetic tweezers, we have performed single-molecule measurements of the unwinding activity of DnaB in which the dsDNA is destabilized by force applied to either the occluded or encircled strand. Based on measurements of the velocity of the motor as a function of force applied to the occluded strand, we conclude that DnaB does not unwind dsDNA with a ''passive'' mechanism, i.e. it does not simply rely on thermal fluctuations to open proximal basepairs before stepping. We also present preliminary data of DnaB activity with force applied to the encircled strand, which probes DnaB's possible mechanisms for binding and translocation along ssDNA. Finally, we will report on the effects of the helicase loader DnaC and the primase DnaG on DnaB activity.

2138-Pos Board B108
Structural Transitions Of a Helicase-Partial Duplex DNA Complex during ATP Hydrolysis Cycle
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Helicases are ATP-dependent enzymes that use the structural changes induced by ATP binding, hydrolysis and release to unwind double stranded nucleic acids. We have studied the structural transitions of partial duplex DNA bound *E-coli* Rep helicase monomer by in vitro single molecule Förster resonance energy transfer (FRET) methodology. Constrained triangulation procedures were applied globally on FRET measurements from eight Rep mutants, donor labeled at different residues, and three DNA substrates, acceptor labeled at different nucleotides on duplex, to study these conformational states. A total of 96 different measurements were performed and used in the triangulation analysis. Such over-sampling reduces the likelihood of a single site with unusual photophysical properties to negatively impact the results. Our results show that binding of ATPγS to Rep induces a large conformational change which is then reversed in two approximately equal steps during ATP dephosphorylation (ATP to ADP.Pi transition) and ADP release. We do not observe a significant conformational change upon phosphate release (ADP.Pi to ADP transition). The large conformational change upon ATPγS binding is consistent with the rotation of the Rep domains in a direction that brings them closer to the duplex. In addition, we show that Rep has a preference to bind to ssDNA/dsDNA junction compared to the other sites along the ssDNA. Finally, we show that Rep remains in the closed conformation during all ATP hydrolysis intermediates when bound to the vicinity of ssDNA/dsDNA junction. Our studies not only reveal the structural transitions of Rep helicase-partial duplex DNA complex during ATP hydrolysis cycle but also demonstrate the potential of triangulation analysis as a versatile single molecule technique for probing structural information in physiological conditions.

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Deciphering the Mechanism of RNA helicase eIF4A in Translation Initiation
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Eukaryotic initiation factor eIF4A is a prototype protein of the DEAD box family of RNA helicases, and is part of the translation initiation complex eIF4F [1].eIF4A binds to the 5′ cap of mRNA and unwinds structures in the 5′-untranslated regions of mRNAs in ATP dependent manner. Although eIF4A has been studied extensively by classical bulk biochemical methods, a direct, unambiguous measurement of its helicase activity and its processivity has not been reported. Here, we use single molecule fluorescence assays to visualize its binding to RNA and melting secondary structures in RNA. Specifically, FRET efficiency dynamics is used to explore the binding location of eIF4A and its unwinding function. Our single molecule studies show that eIF4A has higher binding affinity towards the duplex site. We seek to elucidate any elementary steps and kinetic mechanisms involved with eIF4A unwinding of RNA. Furthermore, we selectively target eIF4A activity with small-molecule inhibitors acting in opposite manners[2]. The dynamics of stimulation and inhibition of eIF4A activity by *pateamine* and *hippuristanol* are measured at the single molecule level. The results will provide insight into the eIF4A's helicase activity and will distinguish between passive versus active unwinding mechanism. Our long-term goal in this project is to decipher the role of the initiation complex eIF4F in ribosomal recruitment, and develop methods to control this process. Then, we will probe eIF4A activity in the presence of other initiation (co)factors.
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2140-Pos Board B110
PcrA Helicase ATPase Mechanism: RepD Modulation During Unwinding
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Helicases catalyse the unwinding of double stranded DNA or RNA for a variety of functions through various mechanisms. The monomeric, bacterial helicase PcrA is well characterized: it translocates ssDNA with discrete steps of one base per ATP. The ATPase activity is enhanced by DNA and the cycle includes a rate limiting cleavage step, followed by rapid phosphate release.
PcrA and the plasmid encoded replication initiator protein, RepD, act together during replication of some plasmids, containing antibiotic resistance. RepD is a dimer and binds to a specific origin sequence (*ori*) containing inverted complementary repeat (ICR) elements. ICRIII provides affinity and plasmid specificity, whereas ICRII is conserved amongst *ori* family members and contains the nick site for initiation. RepD binds to ICRIII and then nicks at a specific site in ICRII exposing a single-stranded region. PcrA helicase then binds to the ssDNA and begins unwinding. RepD has been shown to increase PcrA helicase activity; in the absence of RepD PcrA is a poor helicase. Measurements have shown that unwinding occurs at a reduced rate but the coupling ratio is unaltered.
We have analyzed the mechanism of the PcrA ATPase cycle with ssDNA and extended this to include probing how DNA junctions and RepD affect this cycle. The individual rate constants were determined to see how RepD modulates the ATPase rate. This included utilizing the fluorescent ATP analogue mantATP to monitor fluorescence intensity in rapid-reaction, kinetic experiments, allowing the initial binding and release kinetics to be explored. The analogue was further used for analysis of the hydrolysis step using quenched-flow measurements. Additionally, the fluorescent phosphate binding protein (MDCC-PBP) independently measures the phosphate release step.

2141-Pos Board B111
Single-Molecule Studies Of ATP-Dependent Restriction Enzymes
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